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NITRILE-METABOLIZING BACTERIAL STRAINS ASSOCIATED WITH MUNICIPAL WASTE TIPS IN THE LAGOS METROPOLIS, NIGERIA

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Abstract

Cyanide is one of the dominant pollutants in the environment. This study aimed at exploring the potential of microbes in the detoxification of cyanogenic substances. *Bacillus* sp. WOD8 KX774193 and *Corynebacterium* sp. WOIS2 KX774194 strains were isolated from solid waste leachates. The doubling times of strain WOD8 and strain WOIS2 when grown on glutaronitrile and benzonitrile (without supplementing glucose) were 12.2 and 7.86 d (specific growth rate, μ: 0.057 and 0.088 d^{-1}) and 15.75 and 13.33 d (specific growth rate, μ: 0.044 and 0.052 d^{-1}) respectively. Also, strains WOD8 and WOIS2 grew on glutaronitrile and benzonitrile (with supplementing glucose) with doubling times of 9.76 and 7.62 d (μ: 0.071 and 0.091 d^{-1}) and 10.5 and 8.15 d (μ: 0.066 and 0.085 d^{-1}) respectively. The results from the present study suggest that the nitrile-metabolizing capabilities of these bacterial isolates can potentially be explored for the degradation and bioremediation of nitrile contamination in the environment.

Introduction

Nitriles are cyano group (-C ≡ N) containing organic compounds with the general formula RC ≡ N. These compounds are cyanide-substituted carboxylic acids and are produced naturally as well as synthetically. These

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are found naturally in plants, bone oils, insects, and microorganisms (SMITH 1965, DIGERONIMO and ANTOINE 1976, MUKRAM et al. 2015) and it has been proved that microorganisms can also synthesize them (KNOWLES 1976, BANERJEE et al. 2002). The synthetic nitriles have extensively been used in the manufacture of solvents, extractants, pharmaceuticals and drug intermediates (BANERJEE et al. 2002). Besides, they have also been used in the manufacture of herbicides such as dichlobenil (2,6-dichlorobenzonitrile), bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) (ASHTON and CRAFTS 1973, MUKRAM et al. 2015), the synthesis of polymers and plastics, and are widely used as organic solvents (HENAHAN and IDOL 1971, MUKRAM et al. 2015). Several studies on the application of nitrilases in chemical synthesis have been carried out, since the discovery of first nitrilase in the early 1960s (THIMANN and MAHADEVAN 1964, GONG et al. 2012). The common causes of nitrile entry into the environment include effluents from industrial processes either engaged in nitrile production or processing (WYATT and KNOWLES 1995, RODRIGUEZ 2014), accidental spillage of nitrile from the storage tank (DESHKAR et al. 2003), the use of nitrile compounds as chemical herbicides (VOSAHLOVA et al. 1997) and processing of shale deposit for oil extraction may lead to significant contamination of air, soil, and groundwater (HAWTHORNE et al. 1985, AISLABIE and ATLAS, 1988, RODRÍGUEZ 2014). Therefore, the removal of nitrile from industrial effluents and contaminated places should be of prime importance. Nevertheless, nitrilases play a key role in the bioremediation of hazardous nitriles from the contaminated air, soil, and water systems. Meanwhile, nitrilase-mediated bioremediation is an efficient method for scavenging highly toxic nitriles from environmental wastes and contaminants contaminants (MARTINKOVA et al. 2009, Vesela et al. 2010). The present investigation describes the isolation and characterization of two tropical bacterial species capable of metabolizing glutaronitrile and benzonitrile and a variety of other cyanogenic molecules.

Materials and Methods

Sample collection

Solid waste samples were collected from the dump sites at two locations, Olusosun, Ojota (Coordinates: N 6°29'21.8"; E 003°23'29.3") and Oke-Afa, Isolo (N6°27'11.0002"; E3°23'44.9999") in sterile sample bottles, properly labeled and stored at 4°C, and processed within 24 h. Figure 1 shows the satellite view of dump sites and sampling points.

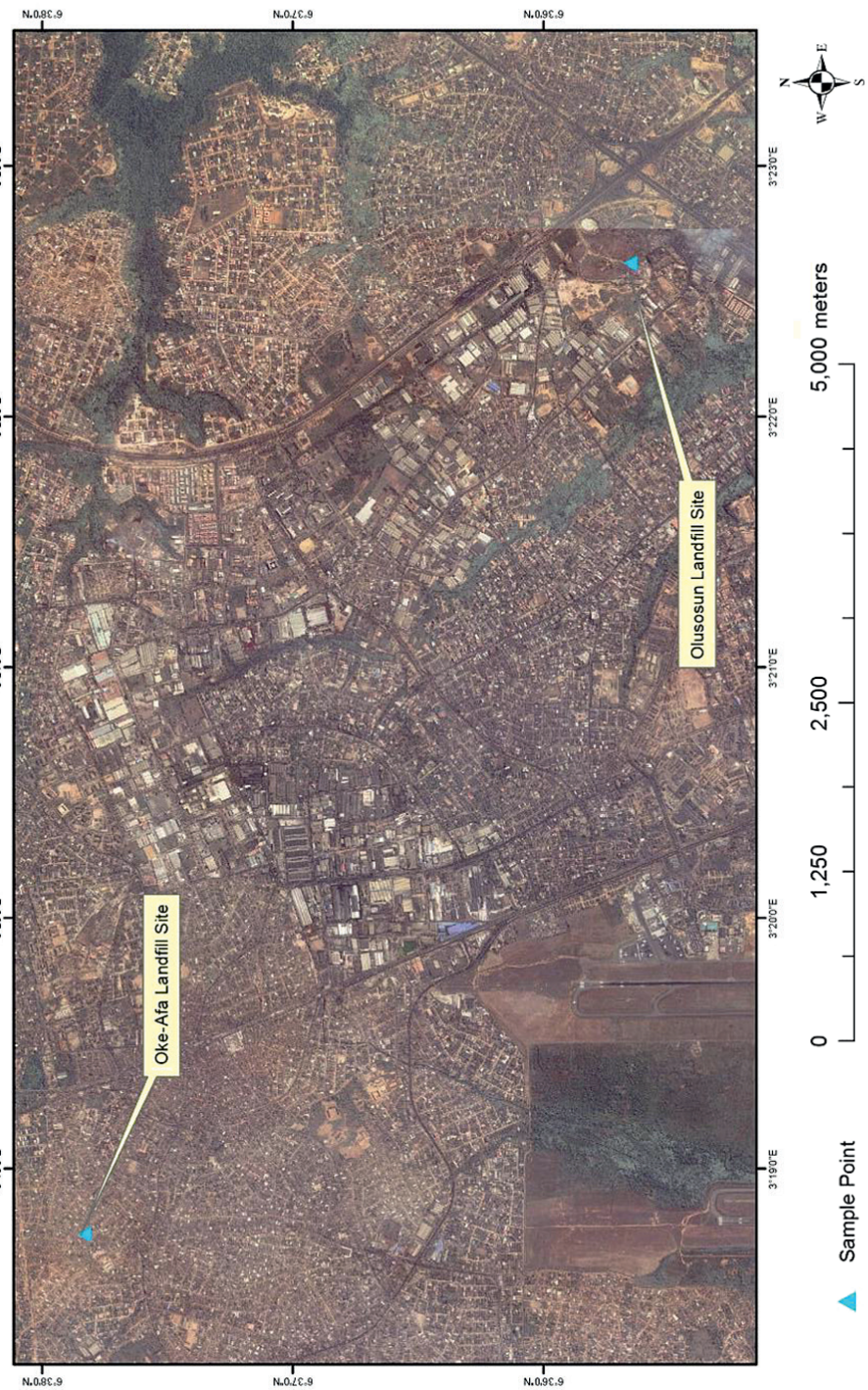

Fig. 1. Satellite image of Olusosun and Oke-Afa Landfill sites in Lagos State, Nigeria

Culture conditions and microbial bacterial enrichment

The bacteria capable of metabolizing glutaronitrile as the sole carbon and nitrogen source were isolated from solid waste leachates by selective enrichment culture technique (SANTOSHKUMAR et al. 2011). About 1.0 g of each of the solid waste samples was suspended in 50 ml of mineral salts medium (MSM) (K_2HPO_4 2.5; KH_2PO_4, 2.0; $MgSO_4.7H_2O$, 0.5; $MnSO_4.4H_2O$, 0.1; $CaCl_2.2H_2O$, 0.06; $FeSO_4.7H_2O$, 0.1; $Na_2MoO_7.2H_2O$, 0.006) supplemented with glutaronitrile (0.2% $^{v}/_{v}$) in a 250 ml Erlenmeyer flask. The flasks were incubated in an orbital shaker (180 rpm) at 32 °C for 7 days. For further enrichment, 5% inoculum was transferred to fresh MSM containing the same concentration of glutaronitrile. After several repeated subcultures, the culture was streaked on MS agar plates containing glutaronitrile. The colonies that grew on agar plates containing glutaronitrile but not on control plates (without glutaronitrile) were selected for further identification. The purity of the culture was checked periodically by plating on agar plates.

Cultural, morphological characteristics and microscopy

The cultural attributes of the isolates were observed visually on nutrient agar plates using a magnifying lens. Cellular morphology was observed using a light microscope. The fresh culture samples were used for the microscopy. The samples were sub-cultured on a fresh nutrient agar plate, smeared and then Gram-stained as described by OGUNYEMI et al. (2010). They were examined for Gram's staining status and other characteristics by using a compound microscope (Hitachi S-3500N model, ThermoNaran, Hitachi technologies, Schaumburg, Illinois, USA).

Biochemical characteristics

Various biochemical assays such as catalase reaction, oxidase, urease, indole, citrate utilization, nitrate reduction, methyl-red-Vogues Proskauer reaction and sugar fermentation were carried out as described by LANYI (1987). The pure cultures of the bacterial isolates were identified according to the identification criteria of Bergey's manual of determinative bacteriology (HOLT et al. 1994).

VITEK identification system

The available identification system was applied to the two (2) isolates obtained and the assays were performed according to the manufacturer's recommendations. VITEK 2 fluorescent card assay were carried out on the

two isolates and the results with very good identification of species of *Corynebacterium* sp. strain WOIS2 and *Bacillus* sp. strain WOD8 were subjected to 16S rRNA gene sequencing.

16S rDNA gene sequencing

The genomic DNA of the strains was extracted and purified following standard protocol for bacterial genomic DNA preparations using Jena Bioscience DNA preparation kits (Germany). 16S rDNA was amplified by Polymerase Chain Reaction (PCR) (94°C for 5 min, 30 cycles consisting of 94°C for 30 s, 55°C for 30 s, 72°C for 90 s followed by a terminal incubation at 72°C for 10 min) using universal 16S rDNA forward 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and reverse 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') primers. The quality of the PCR amplified DNA segments where check by electrophoresis on a 1.5% agarose gel stained with ethidium bromide, using 100 bp DNA marker (Promega, USA) as DNA standard, Millipore water (blank) was used as negative control. The gel was run for 80 min at 100 V, and the amplified products were observed and imaged by Kodak fluorescent imaging equipment, model IS 4000R (Kodak image station, care stream molecular imaging health Inc. Rochester, NY, USA.). Furthermore, the PCR amplified product was purified and the nucleotide sequence was determined with an automated sequencing apparatus (ABI PRISM 377, PE Biosystems Inc.). The 16S rDNA sequences of the strains were searched for homology with the sequences in public databases using the BLAST search program (http://www.ncbi.nlm.nih.gov/) to find closely related bacterial 16S rDNA gene sequences. The generated sequences were deposited in the National Centre for Biotechnology Information (NCBI) nucleotide sequence database as accession numbers KX774193 and KX774194. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 (TAMURA et al. 2007). Neighbor-Joining method was used to construct the phylogenetic tree.

Growth and substrate utilization studies

About 1.0 ml of 48 h grown pure isolate was inoculated in a 250 ml conical flask containing mineral salts medium (MSM 100 ml, pH 7.2, nitrile 0.2% v/v) and incubated at 30°C and 150 rpm. The aliphatic, as well as aromatic nitrile compounds, were tested for their abilities to support the growth of the nitrile-metabolizing bacteria. In order to determine the effect of supplementing glucose with glutaronitrile and benzonitrile in MSM for enhancing the growth of strains of WOD8 and WOIS2, a comparative growth analysis of strains was performed using mixture of each of

the nitrile (glutaronitrile and benzonitrile) and glucose (0.5% w/v). The culture broths were sampled at an interval of 48 h and tested for nitrilase activities over a period of 12 days. For each substrate, two sets of controls (the uninoculated: MSM with the substrate and the inoculated MSM with glutaronitrile and glucose and MSM with benzonitrile and glucose; without any substrate, were put in place to monitor the growth rate of each isolate and to rule out contamination. The bacterial growth was determined by recording the turbidity of the growth medium against the controls in UV-visible spectrophotometer (Thermo Fisher Scientific, USA) at 600 nm. The hydrolysis of glutaronitrile and benzonitrile was measured using spectrophotometer estimation of ammonia release at 630 nm (GUPTA et al. 2010, ALMATAWAH et al. 1999). The release of ammonia is an indicator of cyanide cleavage which simultaneously increases the pH of the medium.

Nitrilase assay

Culture filtrates collected at 48 h intervals from each of the culture flasks were centrifuged (10,000 g, 4°C, 10 min). The supernatants were used as the source of enzymes. The reaction mixture (3.0 ml) comprised of culture supernatant (1.0 ml), glutaronitrile (1.0 ml) and 1.0 ml phosphate buffer (0.2 M, pH 7.2). Nitrilase activity was measured as described earlier (GUPTA et al. 2010, ALMATAWAH et al. 1999) by monitoring the production of ammonia using a UV-visible spectrophotometer at 630 nm for 10 min. One unit of enzyme activity was defined as 1.0 mM of glutaronitrile oxidized per minute. The uninoculated medium was used as the control in all the experiments. All the experiments were carried out in triplicate.

Results

Morphological and biochemical characteristics

By selective enrichment culture technique, eight bacterial strains metabolizing glutaronitrile as the sole carbon source were isolated from solid waste leachates, out of which two were found to grow efficiently on aliphatic as well as aromatic nitrile compounds. Various morphological, physiological and biochemical characteristics of the strain WOD8 and WOIS2 are summarized in Table 1. The light microscopy showed that the strains were Gram's positive (Figure 2). The colonies of strain WOD8 appeared cream-colored, spreading with noticeable smell or pigmentation, while the colonies of strain WOIS2 were white-colored, glossy and non--spreading without any noticeable smell or pigmentation. The strain WOD8 was rod-shaped, while strain WOIS2 was rod-shaped either straight or slightly curved. They were catalase positive and oxidase negative.

Table 1

Morphological, physiological and biochemical characteristics of test strains

Characteristics	WOD8	WOIS2
Gram reaction	+	+
Shape	*R*	*R*
Colour	*C*	*W*
Motility	–	+
Growth	+	+
Catalase	–	+
Oxidase	–	–
Urease	–	+
H_2S	–	+
Indole	–	+
Citrate	+	+
MR	–	–
VP	–	–
Glucose	+	+
Lactose	+	+
Arabinose	+	+
Mannitol	+	–
Maltose	+	+
Putative identification	*Bacillus* sp.	*Corynebacterium* sp.

+ – positive reaction; – negative reaction; *C* – creamy, *R* – rod shaped

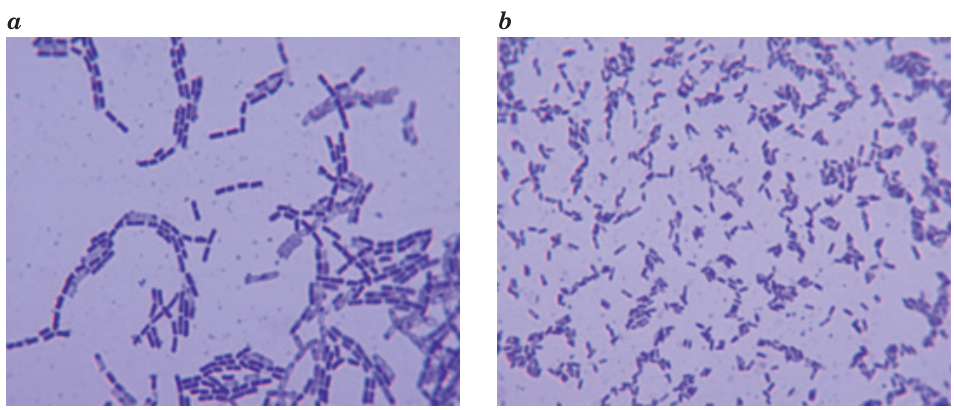

Fig. 2. Light microscopy of *Bacillus* sp. strain WOD8 (*a*) and *Corynebacterium* sp. strain WOIS2 (*b*) at x 1000 magnification. The scale bar indicates 10 μm

The isolates were capable of fermenting glucose, lactose, arabinose, mannitol, and maltose, except the strain WOIS2, which could not ferment mannitol and maltose. They utilized citrate and WOIS2 were found to be positive for H_2S production, urease and indole tests, whereas WOD8 was found to be negative for the same tests. They tested negative to methyl red and the Voges Proskauer test. The isolates were able to produce gas from glucose. The bacterial species were further characterized using VITEK 2 identification system.

Genotypic identities of isolates

The 16S rRNA gene sequences determined the positive identification of isolates. The corresponding genes of strains WOD8 and WOIS2 were sequenced (approximately 1500 bp each) with GenBank accession numbers KX774193 and KX774194, respectively. The homology search showed that the 16S rRNA genes of strain WOD8 had 99% similarity to *Bacillus* sp. Ba29b KU851836, whereas the 16S rRNA genes of strain WOIS2 shared 99% homology with *Corynebacterium* sp. 1031B 12EMannit KU644524 (Table 2).

Table 2
Genotypic identities of nitrile-metabolizing bacterial isolates from amplified sequences of 16S rRNA fragment of genomic DNA

Bacteria strain	Tentative identity	GenBank accession number	Closest strain (s)	% identity	GenBank accession number
WOD8	*Bacillus* sp.	KX774193	*Bacillus* sp. Ba29b	99	KU851836
WOIS2	*Corynebacterium* sp.	KX774194	*Corynebacterium* sp. 1031B12 12EMannit	99	KU644524

The dendrogram constructed for comparing the sequences of some other bacterial strains associated with nitrile degradation in the GenBank indicated that strain WOD8 belongs to the genus *Bacillus,* while WOIS2 belongs to the genus *Corynebacterium* (Figure 3). The dendrogram showed two major distinct clusters; the *Bacillus* group that was closely related and likely to have evolved from same ancestors, and the *Corynebacterium* group that was also closely related but distant from the *Bacillus* group. Strain WOIS2 belongs to cluster one having *Corynebacterium* sp. 1031B 12EMannit KU644524 as member while strain WOD8 belongs to cluster two which had *Bacillus* sp. Ba29b KU851836, as member (Figure 3).

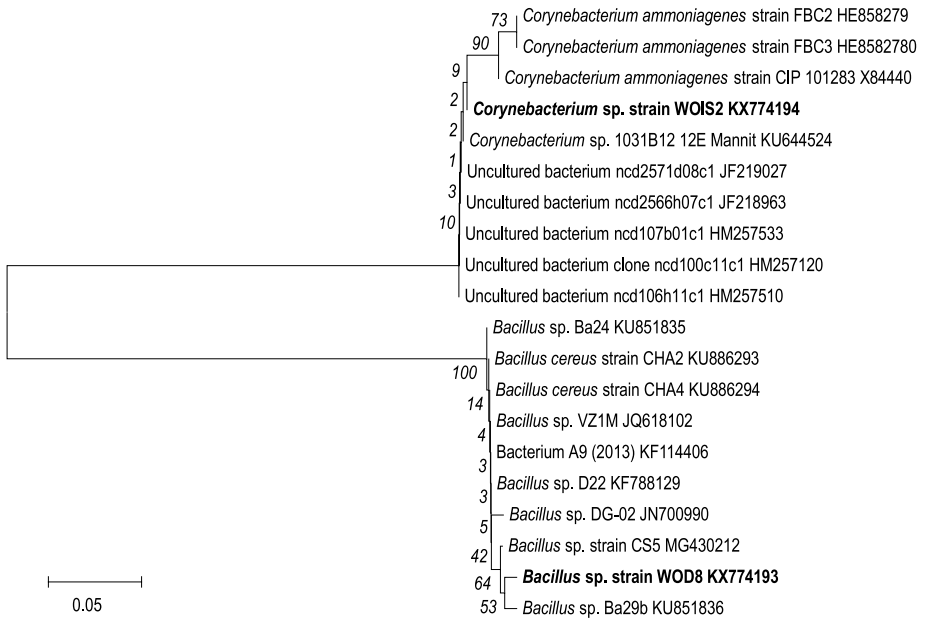

Fig. 3. Phylogenetic tree (dendrogram) of nitrile-degrading *Bacillus* and *Corynebacterium* species based on 16S rRNA sequences using the neighbor-joining method (SAITOU and NEI 1987). Bootstrap test = 1000 replicates. The evolutionary distances were computed using the Kimura[41] parameter method. Analysis involving 20 nucleotide sequences was computed using Mega 6 software

Growth studies of bacterial strains

The growth patterns are shown in Figure 4 and Table 3 revealed the nitrile-metabolizing potential of bacteria. They exhibited exponential growth patterns in the first two days. The highest growth exhibited by *Bacillus* sp. strain WOD8 on glutaronitrile (without supplementing glucose) was 0.552 (O.D 600 nm) with a specific growth rate of 0.057 d^{-1} and doubling time of 12.16 d. Whereas *Corynebacterium* sp. strain WOIS2 recorded highest growth of 0.859 (O.D 600 nm) with a specific growth rate of 0.088 d^{-1} and doubling time of 7.86 d on glutaronitrile (without supplementing glucose).

Table 3

The growth potentials of nitrile-metabolizing bacteria on glutaronitrile and benzonitrile in mineral salts medium

Isolate	μ (d^{-1})				T_d (d)			
	G	*G* + *g*	*B*	*B* + *g*	*G*	*G* + *g*	*B*	*B* + *g*
WOD8	0.057	0.071	0.044	0.066	12.16	9.76	15.75	10.5
WOIS2	0.088	0.091	0.052	0.085	7.86	7.62	13.33	8.15

μ – specific growth rate, T_d – doubling time, *G* – glutaronitrile (without supplementing glucose), *G* + *g* – glutaronitrile plus glucose (with supplementing glucose); *B* – benzonitrile (without supplementing glucose); *B* + *g* – benzonitrile plus glucose (with supplementing glucose)

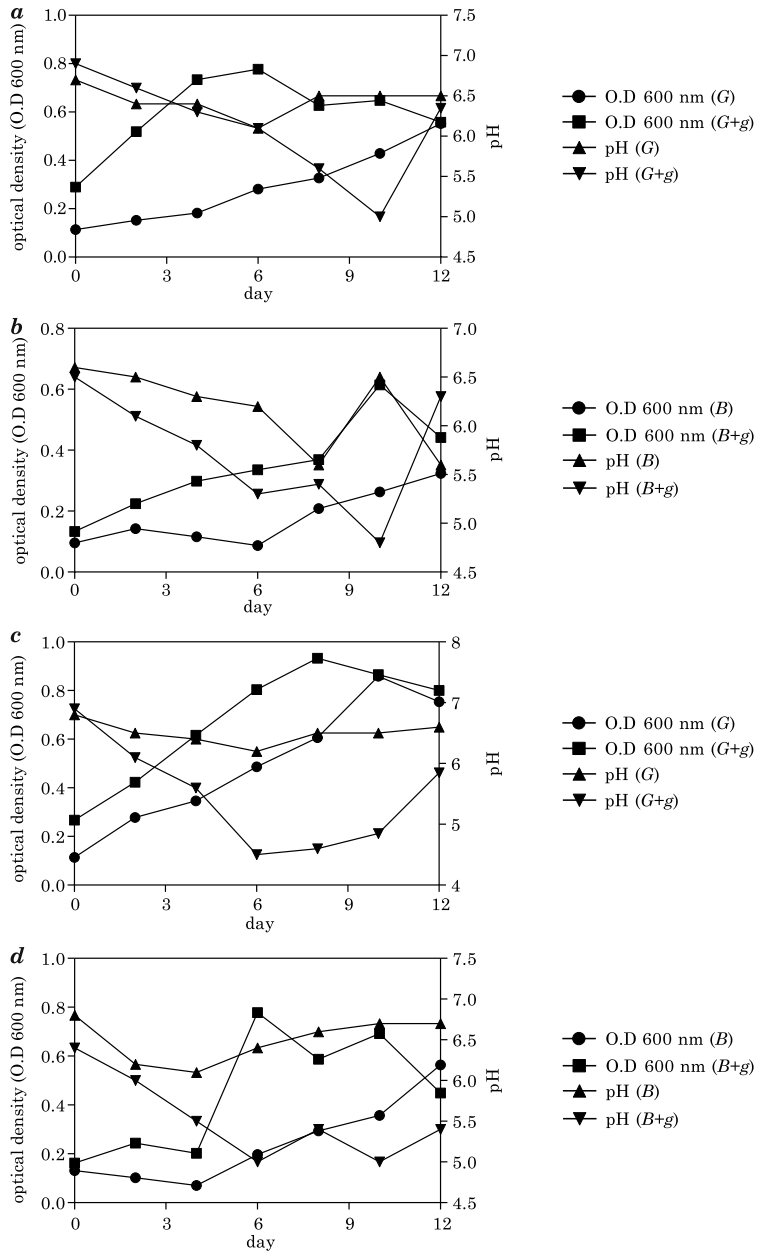

Fig. 4. Growth profiles and pH changes in the culture fluids of *Bacillus* sp. strain WOD8 and *Corynebacterium* sp. strain WOIS2 on glutaronitrile and benzonitrile: *a* – *Bacillus* sp. strain WOD8 on glutaronitrile (with or without supplementing glucose in mineral salts medium); *b* – *Bacillus* sp. strain WOD8 on benzonitrile (with or without supplementing glucose in mineral salts medium); *c* – *Corynebacterium* sp. strain WOIS2 on glutaronitrile (with or without supplementing glucose in mineral salts medium); *d* – *Corynebacterium* sp. strain WOIS2 on benzonitrile (with or without supplementing glucose in mineral salts medium). *G* – glutaronitrile (without supplementing glucose); *G* + *g* – glutaronitrile plus glucose (with supplementing glucose); *B* – benzonitrile without supplementing glucose; *B* + *g* – benzonitrile plus glucose (with supplementing glucose)

While the maximum growth recorded by *Bacillus* sp. strain WOD8 on supplemented glutaronitrile (with supplementing glucose) was 0.777 (O.D 600 nm) with a specific growth rate of 0.071 d^{-1} and doubling time of 9.76 d. whereas *Corynebacterium* sp. strain WOIS2 showed maximum growth of 0.933 (O.D 600 nm) with a specific growth rate of 0.091 d^{-1} and doubling time of 7.62 d on supplemented glutaronitrile (with supplementing glucose). Similarly, *Bacillus* sp. strain WOD8 had highest growth of 0.324 (O.D 600 nm) with a specific growth rate of 0.044 d^{-1} and doubling time of 15.75 d when grown on benzonitrile (without supplementing glucose). Whereas *Corynebacterium* sp. strain WOIS2 recorded highest growth of 0.564 (O.D 600 nm) with a specific growth rate of 0.052 d^{-1} and doubling time of 13.33 d on benzonitrile (without supplementing glucose). While *Bacillus* sp. strain WOD8 had maximum growth of 0.614 (O.D 600 nm) with a specific growth rate of 0.066 d^{-1} and doubling time of 10.5 d on supplemented benzonitrile (with supplementing glucose). Whereas *Corynebacterium* sp. strain WOIS2 had maximum growth of 0.778 (O.D 600 nm) with a specific growth rate of 0.085 d^{-1} and doubling time of 8.15 d on supplemented benzonitrile (with supplementing glucose).

The change in pH

Continuous monitoring of pH revealed a drop in pH (7.2–5.0) and (7.2–6.1) of the medium containing glutaronitrile (with or without supplementing glucose), respectively, inoculated with *Bacillus* sp. strain WOD8 (Figure 4). While on benzonitrile (with or without supplementing glucose), the pH of the culture medium with the same strain, sharply dropped from 7.2 to 4.8 and from 7.2 to 5.6, respectively (Figure 4). Similarly, when *Corynebacterium* sp. strain WOIS2 was grown on glutaronitrile (with or without supplementing glucose), the pH of the culture medium considerably dropped from 7.2 to 4.5 and from 7.2 to 6.2, respectively (Figure 4). While the growth of the same strain on benzonitrile (with or without supplementing glucose), the pH of the culture medium significantly dropped from 7.2 to 5.0 and from 7.2 to 6.1, respectively (Figure 4). From these analyses, it can be concluded that the supplementation of glucose in the culture medium promoted the substrate utilization by the test organisms.

Nitrilase activity

Bacillus sp. strains WOD8 and *Corynebacterium* sp.WOIS2 had maximum nitrilase activities of $3.44 \cdot 10^{-2}$ mg ml^{-1} min^{-1} (day 6) and $2.63 \cdot 10^{-2}$ mg $ml^{-1}min^{-1}$ (day 8) respectively when grown on glutaronitrile (without supplementing glucose) (Figure 5). While optimum nitrilase activities

of $3.94 \cdot 10^{-2}$ mg $ml^{-1}min^{-1}$ (day 6) and $3.89 \cdot 10^{-2}$ mg ml^{-1} min^{-1} (day 8) were obtained by the same strains on supplemented glutaronitrile (with supplementing glucose) (Fig. 5). On the other hand, the maximum nitrilase activities of $1.89 \cdot 10^{-2}$ mg $ml^{-1}min^{-1}$ (day 8) and $2.12 \cdot 10^{-2}$ mg ml^{-1} min^{-1} (day 8) were observed by strains WOD8 and WOIS2 when grown on benzonitrile (without supplementing glucose). While maximum nitrilase activities of $2.52 \cdot 10^{-2}$ mg $ml^{-1}min^{-1}$ (day 6) and $2.56 \cdot 10^{-2}$ mg $ml^{-1}min^{-1}$ (day 10) on were recorded by the same strains on supplemented benzonitrile (with supplementing glucose) – Figure 5. In the previous studies,

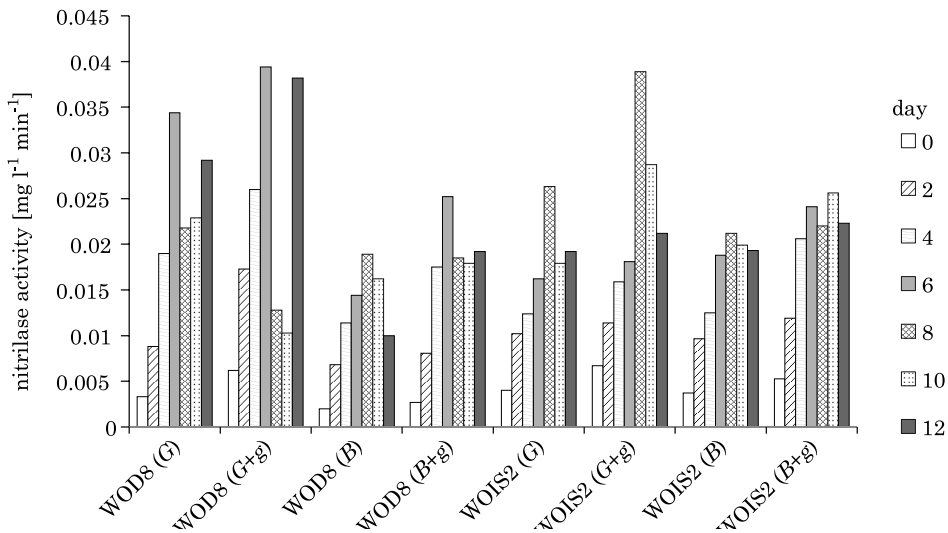

Fig. 5. Niltrilase activities of *Bacillus* sp. (WOD8) and *Corynebacterium* sp. (WOIS2) in growth cultures:
G – Glutaronitrile (without supplementing glucose); *G* + *g* – Glutaronitrile plus glucose (with supplementing glucose); *B* – Benzonitrile (without supplementing glucose); *B* + *g* – Benzonitrile plus glucose (with supplementing glucose)

the selected strains showed varying detoxifying potentials when cyanogenic natural growth substrates were used (OGUNYEMI et al. 2019). In general, the supplementation of glucose with each of the nitrile substrates promoted the nitrilase activity.

Discussion

This study represents the first reported characterization of two nitrile-metabolizing bacterial species from solid waste leachates in Nigeria. The two nitrile-metabolizing bacterial species were isolated from solid waste leachates by selective enrichment technique, which were identified

as *Bacillus* sp. strain WOD8 KX774193 and *Corynebacterium* sp. strain WOIS2 KX774194. The combination of morphological, cultural, and the biochemical characteristics, as well as the 16S rRNA gene sequences identified strains WOD8 and WOIS2 to belong to the genus *Bacillus* and *Corynebacterium,* respectively. The characteristic features of the strains were similar to those reported previously by BRENNAN et al. (2001), YASSIN et al. (2003), LOGAN et al. (2009) and LUDWIG et al. (2007). The result of the phylogenetic tree shows that both the organisms are closely related to each other. *Bacillus* sp Ba29b KU851836 is closest to the strain WOD8, while *Corynebacterium* sp. 1031B12 12EMannit KU644524 is the nearest neighbor of strain WOIS2. The sequence analysis of 16S rRNA gene and unrooted phylogenetic tree showed that *Bacillus* and *Corynebacterium* spp. are likely to have evolved from the same ancestor; however, belong to two different clusters. Although, YAMADA et al. (1980) isolated identified *Pseudomonas* sp. strain K-9; as capable of utilizing only glutaronitrile but failed to utilize other nitrile compounds as growth substrates. Also, DIGERONIMO and ANTOINE (1976) isolated a strain of *Nocardia rhodochrous* that metabolized a selected number of nitrile compounds and their derivatives to carboxylic acids and ammonia. NAWAZ et al. (1989) isolated bacterium capable of utilizing high concentrations of acetonitrile as the sole source of carbon and nitrogen from which had increased growth rates in the media containing range of nitrile compounds including range of nitrile compounds including butyronitrile, glutaronitrile, isobutyronitrile, methacrylonitrile, propionitrile, succinonitrile, valeronitrile, and their corresponding amides, such as acetamide, butyramide, isobutyramide, methacrylamide, propionamide, and succinamide. KAO et al. (2006) and MUKRAM et al. (2015) reported utilization of acetonitrile, propionitrile, benzonitrile, phenyl acetonitrile and butyronitrile by *Klebsiella oxytoca*. *Rhodococcus* sp. MTB5, capable of utilizing benzonitrile as the sole source of carbon and nitrogen, was isolated from a nitrile-contaminated agricultural soil sample by selective enrichment culture technique. In this study, *Bacillus* sp. strain WOD8 and *Corynebacterium* sp. strain WOIS2 isolated were able to utilize both aliphatic (glutaronitrile) and aromatic nitriles (benzonitrile) as a carbon source. BAXTER et al. (2006) explored the potential of a known acetonitrile-metabolizing organism *Rhodococcus* sp. AJ270 for the degradation of acetonitrile and investigated its effects on soil bacterial community, and postulated that the use of such microorganism could play an important role in the detoxification of the toxic compound and thereby, decreasing the risk of environmental contamination. KOBAYASHI and SHIMIZU (1998) proposed that the use of specialized consortia of microorganisms could be a viable alternative to activated sludge for the degradation

and management of toxic chemical wastes. Likewise, KOHYAMA et al. (2006) developed a process for the treatment of acetonitrile-containing wastes by employing two nitrile-degrading microorganisms, *viz., Rhodococcus pyridinivorans* S85-2 and *Brevundimonas diminuta* AM10-C as a source of NHase and amidase, respectively. These strains rapidly established in the nitrile soil community and successfully carried out the bioremediation of nitrile contamination and the addition of acetonitrile significantly affected the composition of the bacterial community in the soil. The drift in pH of the culture medium within 12 days of incubation further confirmed changes in the composition of nitrile substrates possibly degraded by nitrilases (ZHOU et al. 2005). The microbial degradation of nitriles often leads to the production of carboxylic acids and ammonia (HOWDEN and PRESTON 2009). Hence, the formation of acids may probably lead to the reduction in pH levels, which advocates the nitrile utilizing abilities of the strains. Also, in this study, the concentration of ammonia in the residual medium was estimated at different time intervals for the highest accumulation. The prolonged incubation of the culture failed to increase the concentration of ammonia and pH of the medium. The time course study showed a decrease in the concentrations of glutaronitrile and benzonitrile accompanied by the accumulation of ammonia. It was observed that the growth of the selected strains was optimum in glutaronitrile than benzonitrile. Interestingly, the strains displayed substrate dependent nitrilase activities. Furthermore, the bacterial species were grown in cassava effluent and solid waste leachates as natural cyanogenic substrates (sole carbon sources) and they efficiently degraded the cyanogens (OGUNYEMI et al. 2019). The current study claims that the culture conditions and the nature of substrate are the most important factors in the production of nitrilase.

Conclusion

In the present study, the bacterial strains capable of utilizing glutaronitrile and benzonitrile were isolated, characterized, and identified as *Bacillus* sp. strain KX774193 and *Corynebacterium* sp. strain KX774194. These strains were able to grow successfully in aliphatic as well as aromatic nitriles as determined by the growth kinetics. Besides, the strains have the ability to utilize the natural substrate of both cassava effluent as well as solid waste leachates. Therefore, these two strains may be used as the promising tool for the remediation of sites contaminated with both aliphatic and aromatic nitriles. Further research is going on that would focus on deciphering the metabolic pathways and determining the degradative enzymes involved and their metabolic products.

Acknowledgments

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